The Pharma Innovation Journal 2023; SP-12(9): 2175-2178

www.ThePharmaJournal.com

The Pharma Innovation

ISSN (E): 2277-7695
ISSN (P): 2349-8242
NAAS Rating: 5.23
TPI 2023; SP-12(9): 2175-2178

www.thepharmajournal.com
Received: 02-06-2023
Accepted: 12-07-2023

Sushmitha T
P.G. Scholar, Department of Animal Nutrition, CVSc, Rajendranagar, Hyderabad-30 And the Results are The Part of MVSc. of Thesis, Submitted to The PVNRTVU, Hyderabad, Telangana, India

Nalini Kumari N
Professor and University Head, Department of Department of Animal Nutrition, CVSc, Rajendranagar, Hyderabad, Telangana, India

Alexander G
Associate Professor and Head, Department of Animal Nutrition, CVSc, Mamnoor, Warangal, Telangana, India

Harikrishna CH
Professor, Department of Livestock Farm Complex, CVSc, Rajendranagar, Hyderabad, Telangana, India

Corresponding Author:
Sushmitha T
P.G. Scholar, Department of Animal Nutrition, CVSc, Rajendranagar, Hyderabad-30 and the results are the part of MVSc. of thesis, submitted to the PVNRTVU, Hyderabad, Telangana, India

In vitro gas production study to determine the best level of inclusion of tree leaves in chickpea straw based complete feeds

Sushmitha T, Nalini Kumari N, Alexander G and Harikrishna CH

Abstract
The present study was conducted with an aim to study the effect of feeding chickpea straw based complete feed blocks with incorporation of tree leaves on nutrient utilization in native sheep. In order to asses the level of inclusion of two types tree leaves i.e, subabul (*Leucaena leucocephala*) and siris (*Albizia lebbeck*).
In the current study, an *in vitro* gas production study was carried out for chickpea straw based complete diets with 60:40 roughage to concentrate ratio with inclusion of two types of tree leave subabul (*Leucaena leucocephala*) and siris (*Albizia lebbeck*) at 10, 20 and 30 percent replacing concentrate portion to arrive at the best level of inclusion of tree leaves.

Keywords: *In vitro* organic matter digestibility and metabolizable energy

Introduction
Selectivity towards nutritious and palatable feed by animals is another problem in achieving a constant and continuous supply of nutrients to rumen microflora for better utilization efficiency. To obtain improved microbial efficiency from crop residues, the feeding of complete feed has been recommended (Yadav *et al.*, 1990; Sihag *et al*., 1993) [20, 18]. Complete rations facilitate to control ratio of roughage to concentrate, provides uniform feed intake, reduces feed wastage, enhances nitrogen balance and milk production and reduces the cost of feeding (Raut *et al*., 2002; Hundal *et al*., 2004; Lailer *et al.*, 2010) [16, 7, 10]. Inclusion of tree leaves and locally available agro-industrial by-products in complete feeds reduces dependence on costly concentrates (Ganai *et al.*, 2007) [5]. Supplementation of tree leaves have positive impact on rumen microbial growth and digestion resulting in improved growth and enhanced productivity in ruminants (Tessema and Boars, 2004) [19].

Materials and Methods
Diets Formulated for *In vitro* gas production study
Experimental complete diets of seven different types were formulated with chick pea straw as roughage source and concentrate at 60:40 ratio as control (T1) and inclusion of two types of tree leaves Subabul and Sirsi with replacement of concentrate at 10, 20 and 30 percent level to arrive at the best inclusion of two tree leaves through *in vitro* gas production technique. A complete feed was formulated to meet the nutrient requirements of rams (ICAR, 2013) [8] using chickpea straw, maize grain, groundnut cake, cotton seed cake, de-oiled rice bran, molasses, calcite powder, mineral mixture, salt and urea Table which served as a control ration (C). The experimental diets were prepared by replacing concentrate portion with Subabul leaf meal at 10 (S10), 20 (S20) and 30 (S30) and with Siris leaf meal at 10 (A10), 20 (A20) and30 (A30) percent The diets are as followed.

Table 1: Ingredient composition (percent) of complete diets containing Subabul and Siris leaf meals at different levels

Ingredient (%)	C	S10	S20	S30	A10	A20	A30
Chickpea straw	60	60	60	60	60	60	60
Subabul leaf meal	0	10	20	30	0	0	0
Albizia leaf meal	0	0	0	0	10	20	30
Deoiled rice bran	16	12	8	4	12	8	4
Redgram chunni	13.5	10.125	6.75	3.375	10.125	6.75	3.375
Groundnut cake	4.8	3.6	2.4	1.2	3.6	2.4	1.2
Cottonseed cake	4	3	2	1	3	2	1
Urea	0.8	0.6	0.4	0.2	0.6	0.4	0.2
Salt	0.4	0.3	0.2	0.1	0.3	0.2	0.1
Mineral mixture	0.4	0.3	0.2	0.1	0.3	0.2	0.1
Calcite powder	0.1	0.075	0.05	0.025	0.075	0.05	0.025
Total	100	100	100	100	100	100	100

In vitro gas studies to determine the best inclusion level of tree leaves in complete diets

Experimental Design: *In vitro* gas production technique (Menke *et al*., 1979 Blummel *et al*., 1997) [21, 3] was used to determine the best inclusion level of tree leaves by the evaluation of the effect of inclusion of tree leaves in chick pea straw based complete diets at different levels on gas volume, *in vitro* organic matter digestibility (IVOMD) and metabolizable energy (ME) production. The substrate was prepared by mixing chick pea straw as roughage source and concentrate at 60:40 ratio as control and inclusion of two types of tree leaves LL and AL with replacement of concentrate at 10, 20 and 30 percent level each to arrive at the best level of inclusion of two types of tree leaves seperately. The amount of substrate used and the volume of incubation medium were 200 mg and 30 ml, respectively.

Preparation of medium mixture solution

The medium mixture solution was prepared by mixing distilled water, rumen buffer solution, macro mineral solution, micro mineral solution, resazurine solution and reducing solution as shown in Table 6. (Menke and Steingass, 1988) [12]. The reducing solution was prepared freshly and added just before incubation. The medium mixture solution was prewarmed to 39 ºC and bubbled with carbon dioxide after addition of reducing solution.

Rumen liquor collection

The rumen liquor was collected from the sheep with help of stomach tube fitted with vacuum pump before morning feeding. The sheep was maintained by feeding chickpea straw based complete feed (2kg in equal proportions at 9:30 AM and 2:30 PM). The complete feed composed of chickpea straw, maize grain, groundnut cake, cotton seed cake, de-oiled rice bran, red gram chuni, molasses, urea, calcite powder, mineral mixture and salt. The rumen liquor was collected into a prewarmed thermo flask, and brought to laboratory immediately. The rumen liquor was bubbled with CO_2 for about 3 min and filtered through four layers of muslin cloth. The required volume of filtered rumen liquor was added to the medium mixture solution which was colourless. The rumen liquor was added to the medium mixture solution in the ratio of 1:2.

Incubation of Syringes and Subsequent Measurements

The incubation of medium containing rumen liquor was mixed thoroughly using magnetic stirrer at 39 ºC under bubbling with carbon dioxide for about 10 minutes. Then 30 ml of incubation medium was dispensed into a prewarmed (39 ºC) syringes using a dispenser and the syringes were shaken gently. The residual air or bubble in the syringes was removed by gentle shaking and upward movement of piston. After recording the initial volume, the syringes were placed in a water bath with facilities for automatic water circulation at 39ºC.The syringes were shaken at hourly interval for first 2 h from the start of incubation followed by every 2 h up to 6 h of incubation. At the end of incubation period (24 h), gas production in all the syringes was recorded.

Calculation of *In vitro* organic matter digestibility and metabolizable energy

ME (MJ/kg DM) content of feed was calculated using equation of Menke *et al*. (1979) [21] as follows

(MJ/kg DM) = 2.20+0.136 GP+0.057 CP+0.0029 CF2

Where,
GP: 24 h net gas production (ml/200 mg)
CP: Crude protein
CF: Crude fat

IVOMD (%) of feed was calculated using equation of Menke *et al*. (1979) [21] as followed:

IVOMD (%) = 14.88+0.889GP+0.45CP+0.0651XA

Where,
XA = Ash content (%)

Results

In vitro gas production study parameters

In vitro study with *Leucaena leucocephala* leaf meal

The *in vitro* gas production was carried out with chickpea straw based complete feeds with inclusion of LL tree leaves at 10.0, 20.0 and 30.0 parts and the results are presented in Table. The gas volume (ml) ranged from 35.16±0.77 to 36.33±0.30 and ME (MJ/kg DM) ranged from 7.63±0.09 to 7.80±0.04 and IVOMD values (%) ranged from 58.79 to 59.25. There were no significant differences (p>0.05) in *in vitro* gas production parameters among all the three levels of LL leaf meal.

In vitro study with *Albizia lebbeck* leaf meal

The *in vitro* gas production was carried out with chickpea straw based complete feeds with inclusion of AL tree leaves at 10.0, 20.0 and 30.0 parts and the results are presented in Table. The gas volume (ml) ranged from 35.75±0.66 to 36.33±0.30 and ME (MJ/kg DM) ranged from 7.73±0.09 to 7.80±0.04 and IVOMD values (%) ranged from 59.36 to 59.91. There were no significant differences (p>0.05) in *in vitro* gas production.

Table 2: Effect of chickpea straw based complete feed blocks with incorporation of LL leaf meal at different levels on *in vitro* gas production parameters

Parameter	C	S10	S20	S30	SEM	P
Gas (ml) volume	36.33±0.30	35.08±0.67	35.58±0.56	35.16±0.77	0.30	0.464
IVOMD (%)	59.91±0.27	58.79±0.60	59.25±0.50	58.84±0.68	0.26	0.451
ME (MJ/kg DM)	7.80±0.04	7.63±0.09	7.71±0.07	7.65±0.10	0.04	0.469

p-value>0.05

C- Control diet; S10-LL leaf meal @ 10%; S20 – LL leaf meal @ 20%; S30 – LL leaf meal @30%

Table 3: Effect of chickpea straw based complete feed blocks with incorporation of AL leaf meal at different levels on *in vitro* gas production parameters

Parameter	C	A10	A20	A30	SEM	P
Gas volume (ml)	36.33±0.30	35.83±0.62	35.75±0.66	35.83±0.77	0.29	0.905
IVOMD (%)	59.91±0.27	59.40±0.55	59.36±0.59	59.44±0.68	0.26	0.885
ME(MJ/kg DM)	7.80±0.04	7.74±0.08	7.73±0.09	7.74±0.10	0.04	0.921

p-value>0.05

C- Control diet; A10 –AL leaf meal @10%; A20-AL leaf meal @20%; A30 – AL leaf meal @ 30%

Discussion

Gas volume, *in vitro* organic matter digestibility (IVOMD) and metabolizable energy (ME) produced during the *in vitro* gas production procedure performed using chickpea straw based complete diets with the inclusion of two types of tree leaves i.e. LL and AL at different levels i.e., 10.0, 20.0 and 30.0 percent were similar (p>0.05).

Protein fermentation did not lead to much gas production (Khazaal *et al.*, 1995) [9]. In addition fibrous constituents also negatively influenced *in vitro* gas production (Melaku *et al.*, 2003) [11]. These values are comparable with findings of (Akinfemi *et al.*, 2009; Edwards *et al.* 2012; Balgees *et al.* 2013) [1, 4, 2] *in vitro* gas production procedure conducted on tree leaves.

Gas production, IVOMD and ME values in the present study are comparable to those values reported by Gemeda and Hassen (2015) [6].

The similar gas volume of the diets with replacement of concentrate with tree leaves might be due to the lowered condensed tannin content of tree leaves used in the experiment. The gas produced in the syringes might largely be due to acetate and butyrate synthesis, and lower gas production might be associated with propionate production. The total gas production, IVOMD and ME values in the present study are similar to those values reported by Rajkumar *et al.* (2015) [15] who also conducted *in vitro* gas production with inclusion of tree leaves i.e. oak tree leaves and Sarkar *et al.* (2016) [17] who performed *in vitro* gas production with complete feed including *Leucaena leucocephala* leaves.

Perera and Perera (1996) [14] found that, up to 30% of the concentrate could be replaced with *Calliandra calothyrsus.* Nouala *et al.* (2006) [13] observed that, the gas volume and IVOMD were similar up to 50% of replacement of concentrate with *Moringa oleifera* tree leaves.

Concussion

Gas volume, *in vitro* organic matter digestibility (IVOMD) and metabolizable energy (ME) produced during the *in vitro* gas production technique performed using chickpea straw based complete diets with the inclusion of two types of tree leaves i.e., LL and AL at different levels i.e., 10.0, 20.0 and 30.0 percent each were similar (p>0.05). Based on the results of *in vitro* gas production study, all the three levels i.e. 10, 20 and 30 percent of inclusion of tree leaves were acceptable. Hence, 30 percent level of inclusion of tree leaves was taken into consideration for the best level of inclusion.

References

1. Akinfemi A, Adesanya A Oand Aya VE. Use of an *in vitro* gas production technique to evaluate some Nigerian feedstuffs. American-Eurasian Journal of Scientific Research. 2009;4(4):240-245.
2. Balgees A, Elmnan A, Elseed AF, Mahala A, Amasiab E. *In-situ* degradability and *in vitro* gas production of selected multipurpose tree leaves and alfalfa as ruminant feeds World's Veterinary Journal. 2013;3:46-50.
3. Blummel M, Makkar HPS, Becker K. *In vitro* gas production-a technique revisited Journal of Animal Physiology Animal Nutrition. 1997;77:24-34.
4. Edwards A, Mlambo V, Lallo CHO, Garcia GW, Diptee MD. *In vitro* ruminal fermentation of leaves from three tree forages in response to incremental levels of polyethylene glycol Open Journal of Animal Sciences. 2012;2(03):142.
5. Ganai AM, Singh PK, Ahmad HA. Importance of complete feed blocks in livestock production Livestock Line. 2007;1:4-7.
6. Gemeda BS, Hassen A. Effect of tannin and species variation on *in vitro* digestibility, gas, and methane production of tropical browse plants Asian-Australasian journal of animal sciences. 2015;28(2):188-199.
7. Hundal JS, Gupta RP, Wadhwa M, Bakshi MPS. Effect of feeding total mixed ration on the productive performance of dairy cattle. Animal Nutrition and Feed Technology. 2004;4:179-186.
8. ICAR. Nutrient Requirements of Sheep, Goat and RabbitIndian Council of Agricultural Research. New Delhi; c2013. p. 5.
9. Khazzal KM, Dentinho T, Riberrio JM, Oerskov ER. Prediction of apparent digestibility and voluntary feed intake of hays fed to sheep. comparision between using fiber component invitro digestibility or characteristics of gas production or nylon bag degradation. Animal Sciences. 1995;61:527-538.
10. Lailer PC, Dahiya SS, Madan Lal, Lal D. Effect of complete feed blocks on growth performance of Murrah male calves Indian Journal of Animal Nutrition. 2010;27:220-223.
11. Melaku SKJ, Peters, Tegegne A. *in vitro* and *in situ* evaluation of selected multipurpose trees wheat bran and lablab Purpurius as potential feed supplements of tef (Eragrostis tef) straw. Animal feed science and

technology. 2003;108:159-179.
12. Menke KH, Steingass H. Estimation of the energetic feed value obtained from chemical analysis and *in vitro* gas production using rumen fluid. Animal Research and Development. 1988;28:7-5 5.
13. Nouala FS, Akinbamijo OO, Adewumi A, Hoffman E, Muetzel S, Becker K. The influence of Moringa oleifera leaves as substitute to conventional concentrate on the *in vitro* gas production and digestibility of groundnut hay. Livestock Research for Rural Development; c2006, 18.
14. Perera ANF, Perera ERK. Use of Callindra calothyrsus leaf meal as a substitute for coconut oil meal for ruminants. Proceedings of the International workshop on the Genus Callindra. Arkansas, USA; c1996. p. 245-250.
15. Rajkumar K, Bhar R, Kannan A, Jadhav RV, Singh B, Mal G. Effect of replacing oat fodder with fresh and chopped oak leaves on *in vitro* rumen fermentation, digestibility and metabolizable energy. Veterinary world. 2015;8(8):1021-1026.
16. Raut RG, Rekhate DH, Dhok AP. Nutrient utilization in goats fed arhar (*Cajanus cajan*) straw based complete feed pellets. Indian Journal of Animal Nutrition. 2002;19:135-139.
17. Sarkar S, Mohini M, Nampoothiri VM, Mondal G, Pandita S. Effect of Tree Leaves and Malic Acid Supplementation to Wheat Straw Based Substrates on *in vitro* Rumen Fermentation Parameters. Indian Journal of Animal Nutrition. 2016;33(4):421-426.
18. Sihag ZS, Rathee CS, Lohan OP. Effect of different binders and feed ingredients on the formulation of feed blocks. Indian Journal of Animal Science. 1993;63:350-353.
19. Tessema Z, Baars RMT. Chemical composition, *in vitro* dry matter digestibility and ruminal degradation of Napier grass (*Pennisetum purpureum* (L.) Schumach.) mixed with different levels of *Sesbania seban* (L.) merr. Animal Feed Science Technology. 2004;117:29-41.
20. Yadav KK, Rathe CS, Lohan OP. Effect of compaction of roughage based complete feed on digestibility and rumen parameters. Indian Journal of Animal Nutrition. 1990;7:27-30.
21. Krishnamoorthy U, Steingass H, Menke KH, Menke KH. Preliminary observations on the relationship between gas production and microbial protein synthesis in vitro. Archiv Für Tierernährung. 1991;41(5):521-526.